

(19/48) and 39.6% (19/48), respectively, although there was no CYPs positive cases in 30 squamous cell carcinomas. The CYPs positive rates in female adenocarcinoma were higher than those in male adenocarcinoma. The CYPs positive rates in early adenocarcinoma were higher than those in advanced adenocarcinoma. The CYPs positive rates in well and moderately differentiated adenocarcinoma were higher than those in poorly differentiated adenocarcinoma. There were positive relationships among CYP1A1, CYP2E1 and Cyp3A expressions in adenocarcinoma. The CYPs expression in adenocarcinoma was independent of tumor p53 alterations.

**Conclusions:** Our results suggest that better understanding of CYP expression in tumors should be useful and essential, 1) to investigate the mechanism of carcinogenesis, 2) to apply CYP expression as tumor marker, 3) to use the knowledge of CYP expression for molecular targeting therapy, 4) for selection of anti-cancer drug.

P2-050

BSTB: Molecular Targets Posters, Tue, Sept 4

### Blockade of neuropilin-1 decreases NSCLC survival by regulating angiogenic and apoptotic gene expression

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**Background:** Neuropilin-1 (NRP-1) is an isoform-specific receptor for vascular endothelial growth factor (VEGF165) and semaphorin3A. Its expression has been reported on a number of tumour cell lines in the absence of the other VEGF receptors, where it potentially mediates VEGF survival signals. In this study we examined the expression of NRP-1 in retrospective lung cancer tissue, its correlation with survival the mechanisms whereby neutralization of NRP-1 inhibits survival in NSCLC cell lines.

**Methods:** A549, SK-MES1, H460 and H647 NSCLC cells were grown in serum depleted media (0.5%) and screened for NRP-1 expression using western analysis and immunocytochemistry. Cell survival / proliferation were determined by BrdU ELISA following treatment with NRP-1 neutralising antibody or recombinant human VEGF. Apoptosis was determined using the multi-parameter apoptosis kit and In-cell Analyser, and also by FACS. Gene alterations following NRP-1 neutralisation in both A549 and SKMES-1 cells were assessed by quantitative PCR arrays and validated by RT-PCR. A panel of retrospective resected lung tumours were stained for NRP-1 expression by immunohistochemistry.

**Results:** A549, SKMES-1 and H647 cell lines were strongly positive for NRP-1 expression, while NRP-1 expression was absent in H460 cells. All three cell lines positive for NRP-1 displayed significantly ( $p < 0.05$ ) reduced survival following treatment with NRP-1 antibody (1 µg/ml) compared to controls (A549 36%, SKMES-1 51%, H647 43%), while no inhibition of survival was observed on H460 cell line (104%). Neutralisation of NRP-1 induced apoptosis in a dose dependent manner, with decreased f-actin filaments and loss of mitochondrial mass potential. QPCR array data implicated a number of genes regulating these effects which were validated by RT-PCR, including downregulation of bcl-2, MMP1, VEGF and integrin  $\alpha 4$ . To further examine these mechanisms we are preparing cell clones with silenced NRP-1 expression using shRNA technology. The efficacy of combining NRP-1 neutralising antibody with low dose chemotherapy is currently under investigation. NRP-1 expression was observed in a variety of human lung

cancers with different histological subtypes; however strongest expression was noted in adenocarcinoma compared to squamous cell cancer.

**Conclusions:** These results implicate NRP-1 signaling as an important survival pathway in lung cancers. Neutralisation of NRP-1 decreased NSCLC survival, inducing apoptosis through mechanisms including downregulation of bcl-2, integrin  $\alpha 4$  and VEGF. NRP-1 expression was particularly noted in lung adenocarcinoma. Combining strategies targeting NRP-1 with conventional cytotoxic chemotherapy may prove useful as new therapeutic strategies for the treatment of NSCLC.

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### Anti-angiogenic Therapy of Human Small-Cell Lung Cancer Cells with Vascular Endothelial Growth Factor Receptor Tyrosine Kinase Inhibitor, ZD6474

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Anti-angiogenic therapy inhibiting VEGFR2 signaling seems to be an attractive treatment of small cell lung cancer (SCLC) because SCLC produces those receptors. It is reported that effects of small molecules such as gefitinib, an EGFR inhibitor, depend on the status of their targets. We studied effects of ZD6474 which inhibits VEGFR2 as well as EGFR on human small cell lung cancer in terms of anti-angiogenesis.

We compared the effects of a VEGFR2 inhibitor, ZD6474, between SCLCs with and without VEGFR2. SBC-1 showed strong VEGFR2 expression by RT-PCR and the expression was not detectable in MS-1-L. Effects of ZD6474 on cell proliferation and VEGF production were studied. VEGF in culture medium was measured by ELISA. These human SCLC cell lines were inoculated to the nude mice (BALB/c-nu/nu). After inoculated tumor became 2x2x2 mm, xenografts were treated with ZD6474, 12.5, 25 and 50 mg/kg/day, by oral administration for three weeks. The tumor volume was compared between that with ZD6474 administration and that without it. After 3-week treatment, tumor specimens were pathologically evaluated using H&E and TUNEL staining. Tumor angiogenesis was studied by counting vessels in the inoculated tumor by staining vascular endothelial cells with anti-CD31 immunostaining.

VEGF concentration in culture medium of SBC-1 and MS-1-L was 0.44 and 0.99 ng/mL after 72 hours of culture with fresh medium without ZD6474, respectively. Addition of ZD6474 did not change VEGF production in SBC-1, however, the VEGF production was increased to be 3.0 ng/mL in MS-1-L cells when 10 µM ZD6474 was added. ZD6474 did not significantly inhibit SBC-1 cell growth, in contrast, 10 µM ZD6474 significantly stimulated the cell growth in MS-1-L cells. In SBC-1 xenografts, tumor volume was decreased by ZD6474 administration in a dose-dependent manner. In MS-1-L, 12.5 mg/kg/day ZD6474 inhibited the tumor growth, however, the tumor growth was stimulated when 50 mg/kg/day ZD6474 was given. MVC in the inoculated tumor was decreased in SBC-1 by ZD6474 administration, in contrast, MVC did not significantly change in MS-1-L by ZD6474. We found that ZD6474 was effective against SCLC producing VEGFR2. However, ZD6474 stimulated SCLC growth by inducing VEGF production and angiogenesis in SCLC without VEGFR2 expression.